1959

The effect of bacterial and mechanical scars of the kidney on its susceptibility to infection, with a note on the experimental production of renal calculi.

Edvardas Kaminskas
Yale University

Recommended Citation

Kaminskas, Edvardas, "The effect of bacterial and mechanical scars of the kidney on its susceptibility to infection, with a note on the experimental production of renal calculi." (1959). *Yale Medicine Thesis Digital Library*. 2762.
http://elischolar.library.yale.edu/ymtdl/2762

THE EFFECT OF BACTERIAL AND MECHANICAL SCARS OF THE KIDNEY ON ITS SUSCEPTIBILITY TO INFECTION. WITH A NOTE ON THE EXPERIMENTAL PRODUCTION OF RENAL CALCULI

Edvardas Kaminskas

1959

THE EFFECT OF BACTERIAL AND MECHANICAL SCARS OF THE KIDNEY ON ITS SUSCEPTIBILITY TO INFECTION.

With a Note on the Experimental Production of Renal Calculi.

by

Edvardas Kaminskas

B.A. Seton Hall University 1955

A Thesis Presented to the Faculty of the
Yale University School of Medicine
in Candidacy for the Degree of
Doctor of Medicine

Department of Internal Medicine
Yale University School of Medicine

1959

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr. Lawrence R. Freedman for his aid and encouragement in the design, the execution, and the report of this work; to Mrs. Joanne Norton and to Mrs. Elizabeth Maloney for their technical assistance, and especially to the latter for the constant moral support and good cheer. The author appreciates Dr. Paul B. Beeson's permission to use his laboratory.

TABLE OF CONTENTS

RESULTS

TABLE OF ILLUSTRATIONS

INTRODUCTION

De Nevasquez produced pyelonephritis in a rabbit by the intravenous inoculation of Micrococcus pyogenes var. aureus (Oxford strain). After the lesions had healed, an intravenous inoculation of Escherichia coli and Pseudomonas pyocyanea, organisms which are usually incapable of infecting the kidney, resulted in infection. In all cases this infection was localized to nephrons previously obstructed by the scars resulting from the staphylococcal infection. Two types of lesions had been originally produced by the staphylococcus. One was an infected wedge-shaped area with the apex in the medulla and the base in the cortex. The other was a disc-shaped abscess in the medulla without any spread. Healing of the first resulted in a cortico-medullary scar. The second lesion resulted in a localized scar obstructing the tubules which passed through that region, but without infecting them (2).

The present study was designed to test whether infection with E. coli would after an interval of several months also permit a re-infection of the kidney with E. coli.

The lesion due to the original E. coli infection tested is a wedge of abscesses. Since, however, the staphylococcus also produces medullary obstruction without peripheral spread of infection, it was decided to test in addition the late susceptibility of a kidney with a medullary electrocoagulation injury. Such a lesion has previously been demonstrated to render the kidney exquisitely sensitive to infection with intravenous E. coli (7).

An incidental finding in these experiments was that of a method

for the production of unilateral renal calculi without the use of bacteria.

MATERIALS AND METHODS

Animals.-2000-3000 gram white New Zealand rabbits, housed in individual cages in air-conditioned animal rooms, fed on Purina chow and water.

Bacteria.-Escherichia coli ("Y" strain). This strain was originally isolated from the urine of a patient with pyelonephritis. The organism was "passed" through a series of animals by injecting the culture intravenously into rats with obstructed ureters. One week after inoculation the affected kidney was removed, homogenized, and cultured, and the bacteria obtained were then injected into another aminal. After twelve such animal passages the organism was grown in beef heart infusion broth for 3 hours; 5 ml. portions then being frozen and stored at -20° C. Each week one tube was warmed to 37° C., incubated for 24 hours, then "passed" once more through a rat. The culture recovered, after proper identification, was used for all the experiments during the next week. A loopful of the culture was inoculated into 9.0 ml. of beef heart infusion broth at 37° C. for 4 hours. Tenfold dilutions in 0.85 per cent sodium chloride solution were incubated in agar pour plates to enumerate each inoculum.

Injection of bacteria into the kidney.-The animal was anesthetized with 50 mg. of pentobarbital intravenously, supplemented by ether. The abdomen was shaved with electric clippers, cleansed with 70 per cent alcohol, and opened through a midline abdominal incision 7-10 cm. long. The mesentery and the intestinal tract were enveloped in a sterile saline-soaked sponge, and both kidneys were inspected.

A 27-gauge 13 mm. hypodermic needle and a syringe calibrated to 1/100 ml. were used to inject 0.05 ml. of the bacterial suspension. The needle was inserted perpendicular to the surface of the kidney 1.3 cm. medial to the lateral margin, midway between the upper and the lower poles, to a depth of 1.3 cm. Immediately after the removal of the needle slight pressure with a sterile gauze was applied over the puncture site for one to two minutes. The animal was closed using 000 silk for the peritoneum and the muscular wall and metal clips for the skin.

Intravenous injection of organisms.-0.5ml. of a 4 hour culture of E. coli was injected with a 1 ml. syringe and a 24-gauge needle into an ear vein after shaving and cleansing with 70 per cent alcohol the ear skin.

Removal of the kidney for bacteriological and pathological studies.- The animal was killed with 250-300 mg. pentobarbital. The abdominal wall was cleansed with 70 per cent alcohol, and the abdomen opened through a midline incision. The renal pedicle on each side was clamped with snaps and divided, and the kidneys were lifted out of the perirenal fascia into sterile Petri dishes. With a sterile gauze and a sterile knife a midline longitudinal section of the kidney was made. Half of the kidney was placed in a tube of "Precision" homogenizer and ground in 9 ml. of nutrient broth (Difco) until a smooth suspension was obtained. Serial ten-fold dilutions in nutrient broth were then carried out, and pour plates were made according to the expected number of organisms. Plates were incubated for 48 hours and the numbers of colonies were recorded. The remaining half of the kidney was fixed in 10 per cent formol for microscopic sections, which were stained with hematoxylin and eosin and Masson stains.

Production of the renal scar by electrocoagulation of the renal papilla.- Electrocoagulation injury was produced using a fine insulated wire

attached to one of the electrodes of a Davis-Bovie Electro-Surgical Unit. Approximately 2 mm. at the tip of this wire was uncovered. The instrument was set at 25 volts, and the time allowed for heat coagulation to occur was 2-3 seconds.

The animal was anesthetized with 50 mg. of pentobarbital, supplemented with ether. The abdomen was shaved with electric clippers, cleansed with 70 per cent alcohol and opened through a midline abdominal incision. Either the right or the left kidney (depending on the experiment) was isolated and grounded with a sheet of lead. The cautery needle was inserted perpendicular to the surface of the kidney 1.3 cm. medial to the lateral margin, midway between the upper and the lower poles, to a depth of 1.3 cm. After electrocoagulation the wire was withdrawn, slight pressure with a sterile gauze was applied for a few minutes, and the wound was closed, using 000 silk for the peritoneum and the muscular wall and metal clips for the skin.

N.B. All of the above methods have been described in publications from Dr. Paul B. Beeson's laboratory (3,4,7).

RESULTS

PART I. The effect of bacteriemia on the kidney with healed experimental pyelonephritis.

Papillae of left kidneys of thirty-six rabbits were injected with 2.0 to 4.4 x 10^7 E. coli. Random rabbits opened on the fourth post-operative day were found to have purulent abscesses circumscribing their left kidneys, while their right kidneys were found to be free of lesions. Three and half months later three rabbits (controls) were examined and were found to have each a thin scar surrounding the left kidney and extending through the cortex and the medulla into the papilla. (Fig. 1.). Microscopically, a thin band of fibrous tissue containing a few normal-appearing glomeruli, a few tubules, some dilated, some collapsed, some containing colloid concretions, and some round cell infiltrates were seen in otherwise normal kidneys. The right kidneys were free of lesions. Both kidneys were sterile.

Twelve rabbits (experimental) were inoculated intravenously with 2.6 to 3.1 x 10^8 E. coli three and a half months after the papillary inoculations. Examination four days later showed that all left kidneys had thin cortico-medullary-papillary scars described above with no evidence of infection. The right kidneys were normal. Both kidneys were sterile.

PART II. The effect of bacteriemia on the kidney with healed electro-coagulation injury to the papilla.

Sixteen rabbits that had had needle inoculation of their left kidneys three and a half months previously underwent electrocoagulative injury to their right kidney papillae. Two or four months later they were

they were inoculated intravenously with 1.4 to 3.4 x 10^8 E. coli and examined after four days. The right kidneys of thirteen rabbits (three had died from the operative procedure) showed four types of lesions.

Complete atrophy of the kidney.

The right kidney of one rabbit was approximately half the size of the normal-sized left one. The cortex and the medulla were uniformly thinned and extensively replaced by fibrous tissue; the papilla was totally replaced by fibrous tissue. Microscopically, in the fibrous tissue were seen some normal-appearing glomeruli, a few tubules, most collapsed, and in atrophy, some dilated, some containing colloid casts, focal round cell infiltrates, extensive perivascular fibrosis, but no vascular lesions. In this kidney the injury seems to have been placed at the tip of the papilla, obstructing all the collecting ducts and thus producing a complete atrophy of the kidney.

Extensive parenchymal fibrosis with and without calculi formation.

Three rabbits, which were examined two months after electrocoagulative injury to their right kidneys, and four rabbits, which were examined four months after this procedure, had each a wide irregular band of fibrous tissue circling the right kidney.(Fig.2). In the most severely affected kidney (#724) fibrous tissue had replaced most of the renal parenchyma. (Fig.3). The site of electrocoagulation in the papilla was marked by heavy fibrosis, which extended toward the medulla and the cortex. Microscopically, this area was composed entirely of fibrous tissue with many fibroblasts and proliferating capillaries. Fibrous tissue extending to the cortex contained tubules, some of which were normal, but contained colloid

casts, some of which were dilated and others that were collapsed; normal-appearing glomeruli; vessels, some normal, some showing intimal proliferation, some,occlusion and recanalization; and occasional round cell infiltrates. In some kidneys this band of tissue merged into normal parenchyma; in others (as in #724) it had replaced most of the normal tissue.

Right renal pelves of the four rabbits examined four months after the electrocoagulation procedure were slightly dilated and contained coarse, irregular, hard, smudgy yellow calculi, which were not attached to the pelvic epithelium*. (Fig.4). Pelvic epithelium was intact in most areas; no plaques of calcium could be detected, nor were any calcific deposits seen in other parts of the kidney.

In the three rabbits which were examined two months after the electrocoagulation procedure (and in which renal fibrosis seemed to be less severe) the pelves were normal and free of any deposits.

In these seven kidneys the electrocoagulation injury seems to have been placed in the renal papilla, in this way its healing obstructed the collecting ducts draining large areas of the kidney as well as impaired the vascular supply to these ducts.

<u>Hydronephrosis and fibrosis</u>.

Each of the right kidneys of two rabbits had a grossly dilated pelvis, severely scarred and thinned cortex and medulla with no apparent area in which the electrocoagulation had occured. (Fig.5). No

---

*#722 contained four small calculi 2 x 1 x 1 mm to 3 x 2 x 2 mm in size and some gravel

#723 contained a 4 x 3 x 2 mm calculus and some gravel

#724 contained a 9 x 9 x 5mm calculus

#726 contained an 8 x 6 x 6mm calculus

obstruction at the ureteropelvic junction was observed. Microscopically, fibrosis was diffuse, glomeruli appeared normal, most tubules were either collapsed or dilated with or without colloid casts, spotty round cell infiltrates and perivascular fibrosis were present. The pathogenesis of this lesion is not clear; an extra-parenchymal obstructive factor complicated the process of parenchymal atrophy.

Local parenchymal scar.

The right kidneys of three rabbits had each a thin scar traversing the cortex, the medulla, and the papilla. They were comparable to the left kidneys. The injury seems to have been produced not by electrocoagulation but by the passage of the coagulation wire. The left kidneys of the thirteen rabbits showed each a thin scar described in Part I. Both right and left kidneys were sterile.

PART III. The effect of immunization on the susceptibility of the papilla to infection after electrocoagulative injury.

Three rabbits were inoculated intravenously six times during a two week period with 2.6 to 4.4 x 10^8 E. coli per injection. These and three uninoculated rabbits underwent electrocoagulative injury to their left renal papillae. Four days later all were injected with 2.6 x 10^8 E. coli. Four days after the injection two of the rabbits that have been inoculated with E. coli prior to electrocautery and all three of the uninoculated ones were found to have purulent abscesses in their left kidneys, ~~and~~ which grew out more than 1 x 10^5 E. coli. The abscesses started in the vicinity of the coagulated area and pointed along the tract of the obstructed tubules; the needle tract was not infected. Examination of the third rabbit which had E. coli before electrocoagulation showed a blanched left kidney with extensive subcapsular, cortical and medullary

hemorrhages with an area of coagulation necrosis in the papilla, but without infection. It was thought that the arterial supply of this kidney was thrombosed by coagulation and thus the infecting organisms were unable to reach the kidney.

DISCUSSION

The direct injection of E. coli into the rabbit kidney papilla does not, after an interval of three and a half months, result in susceptibility of this kidney to infection by intravenously injected E. coli. This is not surprising when one looks at the scars produced by the original infection. These are sharply localized bands of fibrous tissue which have not altered the adjacent renal parenchyma.

In view of this, the reported ability of the staphylococcus to make a kidney susceptible to infection must result from some lesion other than the infected wedge of renal parenchyma. It was thought that the medullary scar producing obstructed but not infected tubules might represent this lesion. Therefore, the contralateral renal papillae of the remaining rabbits, that had E. coli injected into their left kidneys, underwent electrocoagulative injury. Scarring achieved by this method was very extensive, gross and microscopic hydronephrosis was significant, the alteration of vascular supply was striking, and there was no evidence of current or past infection. However, this lesion, exquisitely susceptible to infection when acute, did not make the kidney susceptible to infection two to four months later.

It was thought, therefore, that either staphylococcal infection produces a hitherto unsuspected alteration in the renal parenchyma or that the animals had become immune to the E. coli organism following the initial infection. To test the latter hypothesis animals were given injections containing large numbers of E. coli over a two week period. This interval of time was allowed for high antibody titers to develop in other rabbits tested in this laboratory. The immunized

animals as well as normal unimmunized animals were given electrocoagulation injuries to the renal papillae. Intravenous injections of E. coli four days later resulted in each group in acute renal infections. It is felt that, although the role of immunity cannot be precisely defined in these experiments, this immunity did not account for the inability to reinfect the scarred kidneys in this study.

It is not clear, then, what changes in the renal parenchyma that were produced by the staphylococcal infection made the scarred and sterile kidneys susceptible to hematogenous infection by E. coli. De Nevasquez related the ability of certain staphylococcal strains to infect a normal kidney via the hematogenous route to their coagulase producing property (1). This or some of the other extracellular substances produced by the staphylococci may produce changes in the renal parenchyma that persist after the infection is healed and thus make the kidney susceptible to hematogenous E. coli infection. These changes may be present in the extracellular matrix or perhaps in the nature of the fibrous tissue laid down. The latter may favor "trapping" of circulating micro-organisms, as suggested by De Nevasquez (2), or it may, behaving in a different fashion than E. coli infection scars or electrocoagulative injury scars, by peculiar growth patterns lead to conditions (e.g. acute tubular obstruction) conducive to bacterial infection. Further studies are necessary to establish the validity of these hypotheses.

A particularly interesting finding in a group of severely scarred kidneys was that of massive pelvic calculi. These were unilateral, in kidneys that never had been infected, and in rabbits that were on a normal diet. No area of attachment to the papilla was found, and examination of the kidney did not show any subepithelial calcium

plaques, "calcium infarcts of the papilla", or nephrocalcinosis. The pathogenesis of these calculi is uncertain; it is likely that necrotic papillary tissue may have served as a nidus for stone formation. These findings would seem to support the theory advanced by Randall (5,6) in explaining the formation of renal stones in man. It is particularly noteworthy that even these kidneys were not infected when injected intravenously with E. coli.

CONCLUSIONS

1. The direct injection of E. coli into the papilla of a rabbit kidney produces an acute infection. Three and one half months later the only evidence of this past infection is a thin sterile scar. Intravenous injection of E. coli at this time does not result in a fresh acute infection within the kidney. Therefore, the scar of previous E. coli pyelonephritis does not increase the susceptibility of the kidney to subsequent E. coli infection.
2. Two to four months after electrocoagulative injury to the renal papilla the kidney is not susceptible to infection by intravenously injected E. coli. This is remarkable in view of the exquisite susceptibility of this lesion when tested one week after having been produced.
3. It is reported that scarring following a staphylococcal pyelonephritis (in contrast to E. coli pyelonephritis) renders the kidney susceptible to infection by intravenously injected E. coli three to six months after the original infection. Possible explanations for this enhancement in susceptibility are discussed.
4. Susceptibility of renal parenchyma, which has been acutely injured by electrocoagulation, to E. coli infection is not modified by immunization against the organism used to produce the infection.
5. An experimental model for producing unilateral renal calculi is presented.

BIBLIOGRAPHY

1. De Nevasquez, S. Experimental Pyelonephritis in the Rabbit Produced by Staphylococcal Infection. J. Path. Bact. 1950. 62:429.
2. De Nevasquez, S. Further Studies in Experimental Pyelonephritis produced by Various Bacteria, with Special Reference to Renal Scarring as a Factor in Pathogenesis. J. Path. Bact. 1956. 71:27-32.
3. Freedman, L.R., Beeson, P.B. Experimental Pyelonephritis IV. Observations on Infections Resulting from Direct Inoculation of Bacteria in Different Zones of the Kidney. Yale J. Biol. Med. 1958. 30:406-414.
4. Guze, L.B., Beeson, P.B. Experimental Pyelonephritis I. Effect of Ureteral Ligation on the Course of Bacterial Infection in the kidney of the rat. J.Expt. Med. 1956. 104:803.
5. Randall, A. The Initiating Lesion of the Renal Calculus. Surg. Gyn. Obs. 1937. 64:201.
6. Randall, A. The Etiology of Primary Renal Calculus. Intern. Abs. Surg. 1940. 71:209-240.
7. Rocha, H., Guze, L.B., Freedman, L.R., Beeson, P.B. Experimental Pyelonephritis III. The Influence of Localized Injury in Different Parts of the Kidney on the Susceptibility to Bacillary Infection. Yale J. Biol. Med. 1958. 30:341.

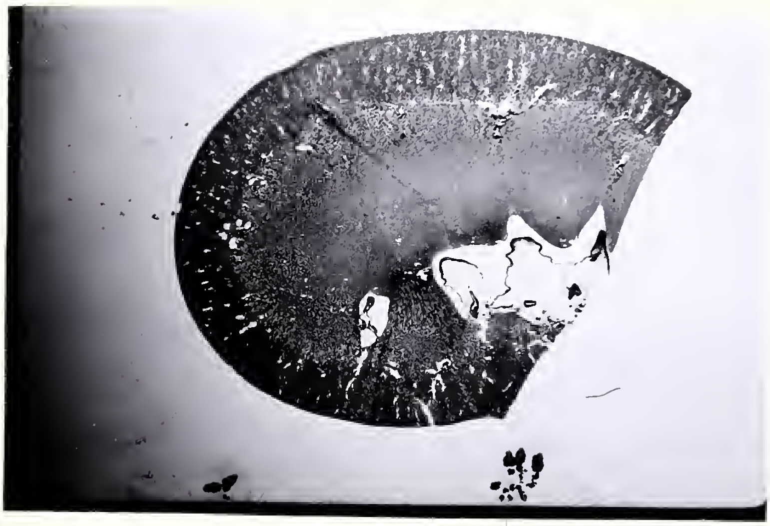

Fig. 1a

Fig. 1b

Figs.1a,1b. Kidneys three and one half months after direct injection of E. coli into the papillae.

Fig. 2

Fig. 3

Figs. 2,3. Kidneys four months after electrocoagulation injury to the papillae, showing extensive parenchymal fibrosis. (Masson's stain).

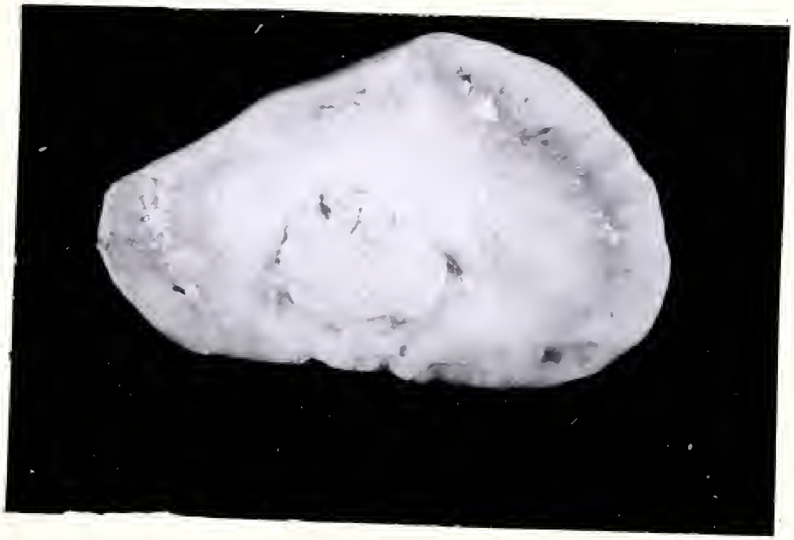

Fig. 4

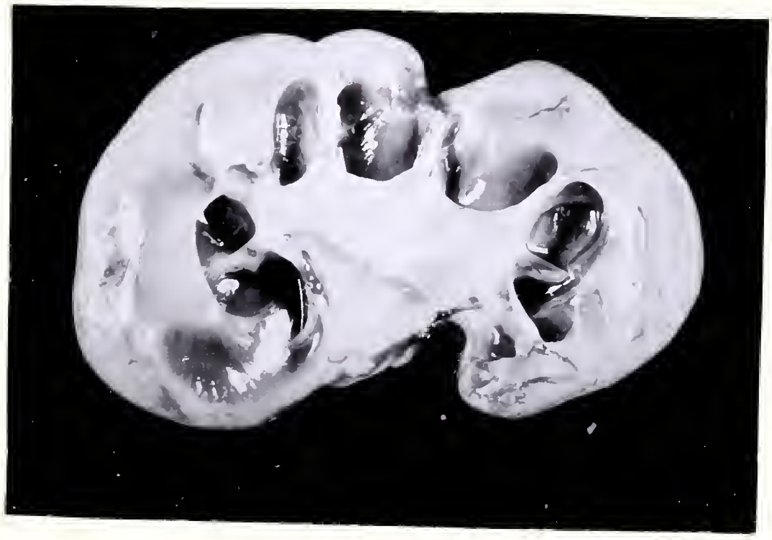

Fig. 5

Fig. 4. Kidney four months after electrocoagulative injury to the papilla, showing a renal calculus.

Fig. 5. Kidney four months after electrocoagulative injury to the papilla, showing gross hydronephrosis and fibrosis.